EXHIBIT A

Gen-Probe Transcription-Mediated Amplification: System Principles

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DNA probe assays have been in use for many years in research laboratories but have only recently become widely used in clinical laboratories. DNA probe assays that target genomic DNA sequences are inherently specific but relatively insensitive because the DNA sequences that they target are present in only one to a few copies per cell. These assays are also relatively complex and lengthy to perform due to the necessity of immobilizing the target DNA on solid supports. In 1986, Gen-Probe introduced the first practical DNA probe tests that were simple and rapid enough for the clinical laboratory to perform. This DNA probe system was the first to be used routinely in clinical laboratories to detect microorganisms such as *C. trachomatis*, *N. gonorrhoeae* and Group A Streptococcus directly in clinical samples. Sensitivity and simplicity of these assays were improved by two Gen-Probe proprietary inventions: the targeting of ribosomal RNA, and a homogeneous, highly sensitive detection system called the Hybridization Protection Assay (HPA). Targeting of rRNA results in a much more sensitive assay because rRNA is present in bacterial cells in many thousands of copies. Sensitivity is further increased by the HPA detection system which utilizes a highly sensitive chemiluminescent signal. Simplicity is achieved because the assay is run in solution phase in a single test tube. No solid substrate or wash steps are required.

Despite the high sensitivity of Gen-Probe's direct assay systems, many types of infectious organisms exist in too few numbers in clinical samples to be detected by this or any other direct DNA probe system. Detection of small numbers of microorganisms requires the use of nucleic acid amplification to increase the amount of nucleic acid present in the sample before detection can take place. Unfortunately, the first amplification systems to be developed were too complex and time consuming to be used routinely in clinical laboratories and have been largely restricted to research laboratories. To overcome these problems, Gen-Probe has developed a nucleic acid amplification method called Transcription-Mediated Amplification (TMA). TMA has been coupled with rRNA targets and HPA in a single, integrated system which is performed in a single test tube. The TMA system is simple and rapid enough to be used in the clinical laboratory and sensitive enough to detect as little as one copy of DNA or RNA in a clinical sample.

The TMA assay system is very similar in methodology to existing Gen-Probe assays, and much of the same equipment is utilized. TMA is an isothermal process and can be performed in a heat block or water bath. No expensive thermocycler is required. The kinetics of TMA are very rapid and billions of RNA amplicons are produced from a single target molecule in less than one hour. TMA can be used with any type of nucleic acid target including rRNA, mRNA or DNA.

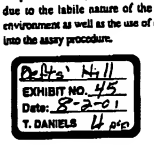
The TMA system is composed of three steps: Sample preparation, amplification, and detection:

SAMPLE PREPARATION

Disruption of the microorganism is necessary to release the target nucleic acid into the assay mixture. Depending on the microorganism to be identified, this is performed either by chemical or enzymatic methods, or mechanical means such as sonication. The released nucleic acid is stabilized, and the cubes are heated briefly at 95°C to denature the target nucleic acid and to inactivate infectious agents. The lysate now contains free nucleic acid that serves as a template for in vitro replication.

AMPLIFICATION

TMA uses two primers and two enzymes: RNA polymerase and reverse transcriptase. One of the primers contains a promoter sequence for RNA polymerase. In the first step of amplification, the promoter-primer hybridizes to the target rRNA at a defined site (Figure 1). Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3' end of the promoter-primer. The RNA in the resulting RNA:DNA duplex is degraded by the RNAse H activities of the reverse transcriptase. A second primer then binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating a double-stranded DNA molecule. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to an exponential expansion of the RNA amplicon. Since each of the DNA templates can make 100-1000 copies of RNA amplicon, this expansion can result in the production of 10 billion amplicons in less than one hour. The entire process is autocatalytic and is performed at a single temperature. Carryover contamination is not a major problem due to the labile nature of the RNA amplicon in the lab environment as well as the use of containment procedures built into the assay procedure.



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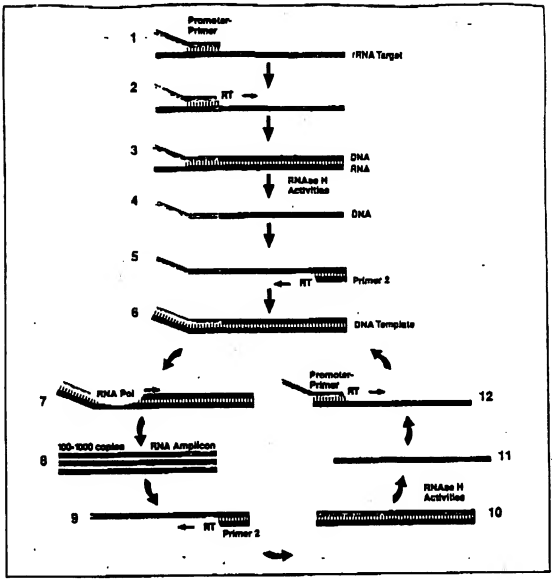

FIGURE 1. Transcription-Mediated Amplification Cycle (TMA):

Step 1: Promoter-primer binds to rRNA target.
Step 2: Reverse Transcriptase (RT) creates DNA copy of rRNA target.
Step 3: RNA:DNA duplex.
Step 4: RNAse H activities of RT degrades the rRNA.
Step 5: Primer 2 binds to the DNA and RT creates a new DNA copy.
Step 6: Double-stranded DNA template with a promoter sequence.
Step 7: RNA polymerase (RNA Pol) initiates transcription of RNA from DNA template.
Step 8: 100-1000 copies of RNA amplicon are produced.
Step 9: Primer 2 binds to each RNA amplicon and RT creates a DNA copy.
Step 10: RNA:DNA duplex.
Step 11: RNAse H activities of RT degrades the rRNA.
Step 12: Promoter-primer binds to the newly synthesized DNA. RT creates a double-stranded DNA and the autocatalytic cycle repeats resulting in a billion-fold amplification.

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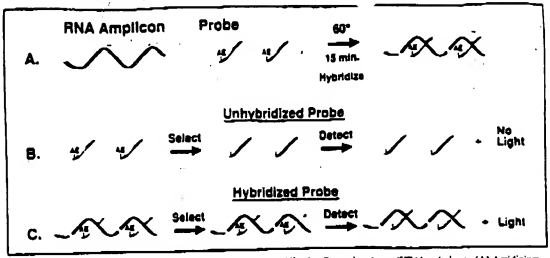

FIGURE 2. Detection of amplicon with DNA probes and the Hybridization Protection Assay (HPA) technique. (A) Acridinium ester (AE)-labeled DNA probes are added and allowed to hybridize to specific target sequences within the amplicon produced in the TMA reaction. (B) Separation of hybridized from unhybridized probes is done by the addition of selection reagent which hydrolyzes the AE on the unhybridized probes. No light is emitted in the luminometer from the unhybridized probes. (C) The AE on the hybridized probes is protected within the double helix and is not hydrolyzed by the selection reagent. Light is emitted and detected by the luminometer.

DETECTION

Detection of amplicon produced by the TMA reaction is performed by the same HPA separation/detection process used in the other Gen-Probe assays over the last 5 years. The process is started by the addition of acridinium ester-labeled DNA probes which specifically bind to the target amplicon (Figure 2). A chemical process is used to distinguish between hybridized and unhybridized probe instead of cumbersome physical separation methods. The final step of the assay is to put the tubes in a luminometer which will automatically inject the proper reagents into the tubes to produce and detect the chemiluminescent signal. This is a homogenous assay format which requires no wash steps that could potentially spread contamination throughout the laboratory. It is particularly suitable for use with target-amplified assays since it offers both absolute specificity and convenience.

ADVANTAGES

The Gen-Probe Amplified assays provide several distinct technological and clinical benefits compared to traditional probe assays and other amplification methods. The most notable include:

- Improved reliability by targeting abundant ribosomal RNA. Since rRNA is present in thousands of copies per cell, the likelihood of initiating amplification is greater than when single copy DNA targets are used. This advantage is very important when organisms are present in low numbers, which is when target amplification methods are most useful.
- Single temperature exponential amplification. The procedure is simple to perform, does not require costly thermocycler equipment, and provides rapid amplification of target sequence present in the sample.
- Primary RNA amplicon. The RNA product of the amplification system is more labile outside the reaction tube than DNA product made by other amplification systems. The risk of laboratory contamination and false positive results is thus substantially reduced.
- Single tube solution format with no wash steps. Reagents are only added to the amplification tube and never removed or transferred. This again minimizes the chance of cross-contamination and false positive results. The single tube, no-wash format also allows for the development of relatively simple instrumentation to automate the amplification and detection steps. Gen-Probe is currently developing automated systems for use with the entire product line.
- Simplicity. With few reagent additions, and HPA detection, the format is user-friendly and familiar to laboratories already using the Gen-Probe DNA probe assays.

The first application of the Gen-Probe TMA system is the Amplified Mycobacterium Tuberculosis Direct Test (MTD) for the detection of *Mycobacterium tuberculosis* in clinical samples. This assay is routinely used in hundreds of laboratories worldwide and provides accurate *same day test results*. Other amplified assays in development are direct assays for *Chlamydia trachomatis*, HIV, chronic myelogenous leukemia (CML), and *Mycobacterium avium* complex.

FURTHER READING

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If you would like more information about Gen-Probe's TMA System please call or write to:

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